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Division-Plane Positioning: Microtubules Strike Back

Two groups have recently developed physical techniques to manipulate the position of the nucleus in fission yeast. Their studies reveal how microtubules confine the nucleus to the cell center, and indicate how the position of the cleavage plane during cell division is coordinated with that of the nucleus.

Manuel Mendoza, Caren Norden and Yves Barral

During cell division the cleavage plane must be positioned correctly between the segregating chromosomes. Animal cells solve this problem by specifying the division plane during mitosis. The mitotic spindle dictates the site of furrowing, ensuring that the contractile ring cleaves the cell into two halves, each containing a complete set of chromosomes [1]. At first sight, the fission yeast *Schizosaccharomyces pombe* appears to use a different mechanism. Fission yeast cells are rod-shaped and divide in the middle. There is a tight correlation between the position of the interphase nucleus and that of the division site. The fission yeast nucleus is maintained at the cell middle during interphase, and mutants that exhibit abnormal nuclear positioning often divide off-center [2].

These and other observations have strongly suggested that, in fission yeast, the position of the interphase nucleus determines that of the cleavage plane. Thus, to understand how the position of the cleavage plane is set, we first need to determine how the nucleus is maintained in the center of the cell during interphase. The answer has long been thought to lie with microtubules. But in the absence of techniques for manipulating the position of the nucleus, the exact role of microtubules has been difficult to address.

Interphase microtubules in *S. pombe* are organized in four to six bundles which span the long axis of the cell. These bundles are anchored by their minus ends at multiple points on the nuclear membrane, so that the highly dynamic plus ends are oriented toward the cell tips [3,4]. Microtubules that contact the cell tips buckle under tension, generating forces capable of deforming the nuclear membrane. It has been suggested that the combined pushing forces of microtubules at opposite cell tips maintain the nucleus in the cell center [4]. Two recent papers [5,6] describe elegant physical approaches to displacing the nucleus of *S. pombe* cells. The results of these studies confirm the role of microtubule pushing forces in nuclear positioning, and bring further insight into the mechanism of cleavage plane specification in fission yeast.

Tolic-Nørrelykke *et al*. [5] used optical tweezers to trap a naturally occurring lipid granule in the fission yeast cytoplasm. By pushing the granule against the nucleus, they could displace it by almost 1 μm in an interphase cell (fission yeast cells are 7–12 μm in length). In most cases, the nucleus returned to the cell center after release from the optical trap, and cells placed the division site in the middle. Visualization of GFP-labeled microtubules showed that the

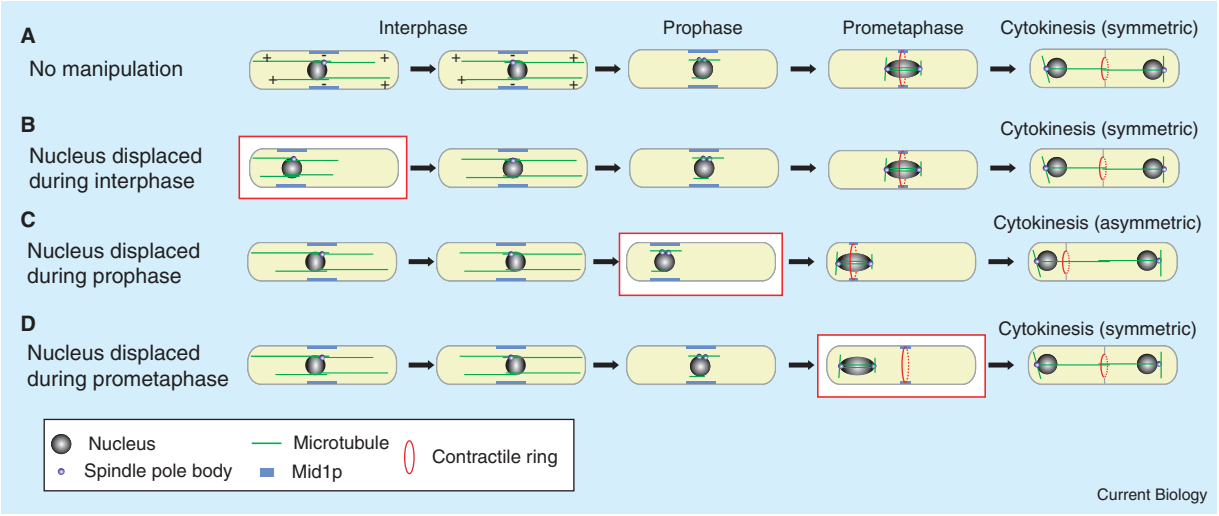

Figure 1. Micromanipulation of the *S. pombe* nucleus during different cell cycle stages, showing the relationship between nuclear position and cleavage plane establishment. Red boxes mark the time of nuclear displacement.

(A) Cytoplasmic microtubules (green) are associated with the nucleus (grey circle) through their minus ends ('–'). Microtubule plus ends ('+') push on the cell tips and maintain the nucleus in the cell center during interphase. Mid1p (blue) accumulates in a broad cortical region overlying the nucleus. Upon spindle formation, cortical mid1p is restricted to a sharp ring, which coincides with the position of actomyosin ring formation (red). Accordingly, cytokinesis and septum formation take place in the cell middle. This leads to symmetric division and two daughter cells of the same length. (B) When the nucleus is displaced during interphase, microtubules push it back to the cell center. Nuclear movement is accompanied by re-centering of cortical mid1p. This results in symmetric cell division, like in non-manipulated cells. (C) During prophase, the duplicated spindle pole bodies (depicted in purple) separate and cytoplasmic microtubules are disassembled. As a result, microtubules cannot correct the position of the nucleus after manipulation. This causes mispositioning of both the mid1p cortical domain and the actomyosin ring, and cells divide off-center. (D) Displacement of the prometaphase nucleus does not affect cleavage plane positioning. The sharp mid1p ring is no longer coupled to the nucleus: the actomyosin ring assembles at the cell middle, even if the nucleus is off-center.

movement of the nucleus towards the center was associated with microtubule pushing on the cell end. Moreover, when microtubules were depolymerized by treatment with thiabendazole, the manipulated nuclei failed to return to the cell center.

Remarkably, displacement of the interphase nucleus in the absence of thiabendazole caused some cells to divide asymmetrically: the cleavage plane was no longer in the cell center but correlated with the position of the displaced nucleus. This demonstrates first, that the position of the cleavage plane is specified by the nucleus; and second, that this specification occurs prior to mitosis. In support of this last conclusion, displacement of the nucleus during prometaphase did not displace the division site. In contrast, moving the nucleus at an earlier stage — during prophase — caused some cells to divide asymmetrically (Figure 1). From these results, Tolic-Nørrelykke *et al*. [5] conclude that the nucleus specifies the division site before prometaphase.

How does the nucleus specify the site of division? Mid1p has been proposed to be a key protein in this process. Mid1p accumulates in the nucleus during interphase and is exported to a broad band at the cell cortex overlying the nucleus, starting long before mitosis [7]. During anaphase, this band condenses into a sharp ring that corresponds to the site of cleavage. Importantly, cells lacking mid1p often divide asymmetrically, even if their nuclei are maintained at the cell center [8–10]. So mid1p may couple the position of the cleavage plane to that of the nucleus. The micromanipulation experiments of Tolic-Nørrelykke *et al*. [5] are consistent with the dynamics of mid1p localization: they show that the position of the cleavage plane is set shortly after the start of mitosis, which is exactly the time at which mid1p transfer to the medial cortex is complete.

Independent experiments by Daga and Chang [6] directly address the relationship between nuclear position and mid1p localization. Instead of optical micromanipulation, these authors used centrifugation to displace the nucleus of *S. pombe* cells. In agreement with Tolic-Nørrelykke *et al*. [5], they found that cells treated with MBC, another microtubule-depolymerizing drug, failed to re-center displaced nuclei, whereas they did reposition their nuclei in MBC-free medium. Moreover, in contrast to cells centrifuged during interphase, which went on to divide asymmetrically, cleavage plane positioning was not affected by centrifugation during mitosis. Daga and Chang [6] found that, during interphase, the position of the cortical mid1p domain followed that of the displaced nucleus. Imaging of mid1p-GFP revealed that cortical mid1p moved back to the cell center together with the nucleus. Thus, the mid1 cortical domain is regulated by the underlying nucleus in a dynamic manner.

Now that the role of the nucleus in determining the position of the cleavage plane is demonstrated,

the next question to be solved is: how does the nucleus specify the 'mid1 domain' at the cell cortex? One possibility is that nuclear export of mid1p, together with diffusion, targets mid1p to the cell cortex overlying the nucleus. However mid1p associates with the cortex, the question is how its cortical localization is maintained at the cell equator.

Double septin rings assemble at the division site in both fission and budding yeast and, at least in the latter organism, they function as diffusion barriers to confine cytokinesis factors to the division site [11]. But double septin rings form only during anaphase in *S. pombe*, which is too late to influence mid1p distribution. Alternatively, a physical link between the nucleus and the cortex may control mid1p localization. A possible candidate is the endoplasmic reticulum (ER), which is formed by membranes extending from the nuclear envelope to the entire cell cortex [12]. Interestingly, recent studies show that, in budding yeast, this organelle is highly compartmentalized [13], indicating the existence of ER diffusion barriers in this, and perhaps other, organisms. Whether mid1p distribution is dependent on ER membranes or other, as yet unidentified structures, is an issue that remains to be addressed.

Beyond advancing our understanding of cleavage plane specification, these studies [5,6] also highlight the role of microtubule networks in maintaining subcellular organization. Many processes involving directed nuclear movements depend on microtubule pulling, as opposed to pushing. For example, migration of the male pronucleus towards the center of *Caenorhabditis elegans* eggs depends on the combined action of microtubules and dynein motors [14], as does nuclear migration into the bud in budding yeast [15]. On the other hand, microtubule-based pushing of an organelle could be the best solution to the problem of finding the geometrical center in a confined space. As has been noted, precise regulation of microtubule dynamics is essential even in this simple case, since microtubules that are too stable would bend around the cell ends resulting in mispositioning of the nucleus [16]. The use of micromanipulation techniques in cells defective in specific aspects of microtubule dynamics promises rapid advances in our understanding of how cells monitor their spatial organization.

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Ecology: Linking Species Diversity and Genetic Diversity

Although there is a great deal of interest in the biological diversity of species and of genes, it is only recently that researchers have begun to investigate the processes that exert parallel influences on these different levels of diversity.

Anne E. Magurran

Population geneticists and community ecologists often ask similar sorts of questions. Both sets of scientists are interested in measuring, and understanding, the number and distribution of biological variants found in nature. Indeed the conceptual links between the disciplines are recognized in the most widely used definition of biological diversity, devised by the Convention on Biological Diversity, which refers directly to 'variability among living organisms' and stresses that this includes diversity within species as well as between species [1]. But despite striking parallels in approach there have been few attempts to examine these levels of diversity simultaneously.